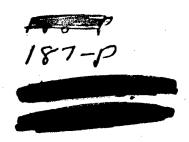
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ANNUAL PROGRESS REPORT

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Dr. B.A. Sobin

Chas. Pfizer & Company, Inc.

Brooklyn, N.Y.

Preparation and Evaluation of Staphylococcal Enterotoxoids

Contract No. DA-49-193-MD-2428



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#### ABSTRACT

1. Preparing Institution:

Chas. Pfizer & Company, Inc., Brooklyn 6, N.Y.

2. Title of Report:

Preparation and Evaluation of Staphylococcal Enterotoxoids

3. Principal Investigator:

Dr. B.A. Sobin

4. Number of pages and date:

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United States Army Medical Research and Development Command, Department of the Army, Washington 25. D.C.

Thirty Staphylococcal enterotoxoids were prepared and evaluated in rhesus monkeys in a range finding study designed to compare toxicities, antibody response, and protection against intravenous challenge with untreated Staphylococcal enterotoxin.

The immunization schedule consisted of three subcutaneous injections of toxoid with adjuvant spaced at intervals of five weeks. Some toxicity (emesis, diarrhea) was observed with most of the toxoid preparations following the first subcutaneous immunization injection. No toxic reactions followed the second and third immunization injections.

Antibody titers, as measured by hemagglutination and gel-diffusion, were generally low after the first immunization injections. Many of the antibody titers rose sharply after the second and third injections.

A number of the Staphylococcal enterotoxoid preparations provided excellent protection in monkeys against intravenous challenge with untreated Staphylococcal enterotoxin 10 1/kg. and 1000 1/kg. three weeks after the third and final immunization injections.

The ten most promising toxoid preparations were selected from the range finding studies for expanded toxicity comparisons, antibody titers, and degree and duration of immunity to intravenous challenge with Staphylococcal enterotoxin.

In the extended evaluation studies to date, Sample #10 (0.2% formalin + 0.15% glycine at 25°C. for 2 weeks) is the most impressive of the toxoid preparations on the basis of toxicity and antigenic response. The final evaluation will depend on the degree and duration of immunity to intravenous challenge with Staphylococcal enterotoxin, correlation of immunity with hemagglutination titers, effect of booster shots, and nature of anamnestic response.

7. Protection against intravenous challenge with Staphylococcal enterotoxin based on immunization with enterotoxoid preparations.

#### ABSTRACT DISTRIBUTION LIST

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#### INTRODUCTION

The establishment of the protein and antigenic nature of Staphylococcal enterotoxin, and the availability of a supply of highly purified material provided the background for the following objectives:

- a. To prepare a number of toxoids of Staphylococcal enterotoxim by treatment with a variety of chemical and physical agents.
- b. To test and evaluate the immunological responses of the various Staphylococcal enterotoxoid preparations in rhesus monkeys.
- c. To select a safe and effective Staphylococcal enterotoxoid capable of conferring upon rhesus monkeys immunity of long duration against the action of a large dose of intravenously administered Staphylococcal enterotoxin.

The construction of an animal facility to house two hundred rhesus monkeys was begun in the early part of May, 1963, and was completed August 15, 1963. Animal experimentation was initiated September 3, 1963.

Working supplies of highly purified Staphylococcal enterotoxin, Lot No. 14-31, were made available on May 28 and June 17, 1963.

A search of the literature was made covering the chemical and physical agents used to prepare toxoids, inactivate microorganisms, and alter proteins. Thirty toxoid treatments were selected for evaluation in a preliminary range finding study comparing toxicity, serologic response, and protection against intravenous challenge with untreated Staphylococcal enterotoxin. Ten Staphylococcal enterotoxoid preparations were chosen from this group for evaluation over a twelve month period with a scheduled termination date of May 30, 1965.

#### MATERIALS AND METHODS

#### TOXOID PREPARATIONS

#### Formalin

5 ml. of the various formalin solutions were added to each of a number of vials containing 10 mg. of highly purified freeze-dried Staphylococcal enterotoxin, Lot No. 14-31. After maintenance under the designated experimental conditions, the vials were stored at -20°C.

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	1.	0.2%	formalin	at 37 c	for 1	week		
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	٦٠			•	" 3	veeks		!
		•	formalin	-				•
	5.	0.84	formalin	at 37°C.	for 1	veek		
	5. 6.	н	**	" "	" 2	veeka		
	7.	rt	. 10	10 17	" ~	weeks		
	•				3	ACCVR		
i			formalin	_				
(	9.	0.24	formalin	0.15%	glycine	at 25°C.	for 1	week
	ó.	"	"	"-7"	H A Table	" -1	7 2	weeks
	-	**	**	**	**	11 11		
1	l.			•			י כ	reeks

#### Hexamethylenetetramine

#### Beta-Propiolactone

- 14. 5 ml. of a chilled 0.2% solution of beta-propiolactone in isotonic disodium hydrogen phosphate was added to each of a number of vials containing 10 mg. of toxin, and held at 37°C. for 2 hours. The reaction was allowed to proceed overnight at room temperature, and then stored at -20°C.
  - 15. Same as above except for change to 1.0% beta-propiolactone.

#### Diethyl Pyrocarbonate

- 16. 5 ml. of a chilled 0.2% solution of diethyl pyrocarbonate in ethanol-water (1:1) was added to each of a number of vials containing 10 mg. of toxin, and stored for 1 week at 5°C. The vials were then held overnight at room temperature, and then stored at -20°C.
  - 17. Same as above except for change to 1.0% diethyl pyrocarbonate.

#### Ethylene Oxide

- 18. 5 ml. of a chilled 0.2% solution of ethylene oxide was added to each of a number of vials containing 10 mg. of toxin. The vials were held at 5°C. for 2 hours, maintained overnight at room temperature, and stored at -20°C.
  - 19. Same as above except for change to 1.0% ethylene oxide.
- 20. A number of vials of toxin, 10 mg./vial, were placed in a vacuum desiccator along with 5 ml. of liquefied ethylene oxide contained in a small Erlenmyer flask. The vacuum desiccator was partially evacuated to allow for the expansion of ethylene oxide vapor. After 24 hour exposure in the ethylene oxide atmosphere, the trace amounts of adsorbed ethylene oxide were removed by exhausting the vacuum desiccator for several hours. The vials were stored at -20°C.

#### Ethyleneimine

21. A number of vials of toxin, 10 mg./vial, were placed in a vacuum desiccator along with 5 ml. of ethyleneimine contained in a small Erlenmyer flask. The vacuum desiccator was partially evacuated to allow for the expansion of ethyleneimine vapor. After 24 hours exposure in the ethyleneimine atmosphere, the vacuum desiccator was exhausted for 24 hours. The vials were stored at -20°C.

#### Methyl Bromide

22. A number of vials of toxin, 10 mg./vial, were placed in a vacuum desiccator along with 5 ml. of liquefied methyl bromide contained in a small Erlenmyer flask. The vacuum desiccator was partially evacuated to allow for the expansion of methyl bromide vapor. After 24 hours exposure in the methyl bromide atmosphere, the trace amounts of adsorbed methyl bromide were removed by exhausting for several hours.

#### Propylene Oxide

23. Procedure identical to that described for ethylene oxide and methyl bromide.

#### Methanol Esterification

24. Two ml. of absolute methanol saturated with dry hydrogen chloride was added to each of a number of vials containing 10 mg. of toxin. After standing for 3 days at room temperature, the vials were taken to dryness in vacuo. Two ml. of sterile distilled water was then added to each vial, the material freeze-dried, and stored at -20°C.

#### Sodium Gluconato

25. 5 ml. of an aqueous 1.0% solution of addism gluconeth was added to each of a number of vials containing 10 mg. of toxin. After holding for 4 weeks at 37°C., the vials were stored at -20°C.

#### Intraviolet Light Irradiation

- 26. 250 mg. of toxin was dissolved in water and transferred in a final volume of 125 ml. to a stoppered quartz Erlenmycer flask fitted with a magnetic stirrer, and cooled by running water. The stirred solution was exposed to the rays from an ultraviolet light lamp, short wave (2537 Angstrom units), fixed at a distance of 20 cm., for 1 hour. Two ml. aliquots were transferred to a number of sterile vials, and stored at -20°C.
  - 27. Same as above except for exposure time of 5 hours.

#### Photodynamic Action of Methylene Blue

- 28. 250 mg. of toxin was dissolved in an aqueous solution of methylene blue (1:10,000), and transferred in a final volume of 125 ml. to a stoppered Erlenmyer flask fitted with a magnetic stirrer, and cooled by running water. The stirred solution was exposed to the light from a 100 Watt incandescent bulb fixed at a distance of 20 cm. for 1 hour. Two ml. aliquots were transferred to a number of sterile vials, and stored at -20°C.
  - 29. Same as above except for exposure time of 8 hours.

#### Acetic Anhydride

30. To 250 mg. of toxin dissolved in 5 ml. of saturated sodium acetate solution and cooled in an ice bath, was added with stirring 0.3 ml. of acetic anhydride. After stirring for an hour, the material was carefully transferred to a small cellophane bag, and dialyzed against distilled water in the refrigerator for 48 hours with frequent changes of water. Two ml. portions of the opalescent solution were transferred to each of a number of sterile vials, 4 mg./vial, and stored at -20°C.

#### Adjuvant

A light mineral oil (Drakeol 6VR) and a mannide monoleate (Arlacel A) were mixed in a 9:1 ratio, and sterilized by Seitz filtration.

#### Emulsification

To each vial containing 1 ml. of toxoid solution, 2 mg./ml. was added an equal volume of the mineral oil - Arlacel A solution. Emulsions of the vaccines were prepared directly in the vials by forcing the contents back and forth through a 1 ml. glass tuberculin syringe fitted with a 20 gauge needle. The process was continued until a drop of emulsion expelled through the hypodermic needle into a beaker of cold water retained its shape.

#### Immunization Schedule

#### Range Finding Studies

Pairs of rhesus moneys were immunized with each of the thirty different toxoid preparations. The immunization schedule consisted of three subcutaneous injections spaced at five week intervals. A total dose of 0.10 mg. was given in the first injection. The second and third injections each consisted of a total dose of 0.25 mg. of toxoid.

The thirty pairs of monkeys, previously immunized with the thirty different enterotoxoid samples, were challenged intravenously intravenously three weeks after the third and final immunization injections with untreated Staphylococcal enterotoxin dissolved in 0.02 M phosphate buffer, pH 7.4. One monkey of each pair was challenged at 10 /kg., and the other at 1000 /kg.

#### Extended Evaluation Studies

Twelve monkeys were immunized, in the manner already described, with each of the ten selected toxoid samples. Four monkeys from each set are to be challenged intravenously with untreated Staphylococcal enterotoxin six months after the third and final immunization injections. At the same time, a second set of four monkeys from each group is to be given a booster shot of 0.25 mg. of toxoid. These monkeys, along with the third set of four monkeys from each group, are to be challenged intravenously with untreated Staphylococcal enterotoxin one year after the initial immunization period.

#### Toxicity Determinations

After each injection of Staphylococcal enterotoxin or toxoid, absence or presence of illness (emesis, diarrhea) was noted through a five hour observation period. Death or delayed death with marked weight loss was interpreted as a direct toxic manifestation of the injected material.

#### Serologic Tests

Antibody titers were determined by hemagglutination and gel-diffusion tests as performed at Fort Detrick.

The lowest dilution of sera in the hemagglutination test was arbitraril set at 1:10,000. Titers are expressed as the reciprocal of the highest serum dilution showing gross hemagglutination.

In the gel-diffusion assay, a preliminary qualitative test was run in single tubes with sera diluted 1:10 overlaid with Staphylococcal enterotox 100 v/ml. Samples which gave sharp, demonstrable zones after twenty four hours were assayed in the usual eight tube quantitative test. Titers are expressed as reciprocals of the number.

#### RESULTS

#### Range Finding Studies

Some toxicity (emesis, diarrhea) was observed following the first subcutaneous immunization injection of the Staphylococcal enterotoxoid samples. There were no deaths. No toxic reactions followed the second and third immunization injections.

Antibody titers were generally low or negative after the first immunization injections. Many of the antibody titers rose sharply after the second and third subcutaneous immunization injections.

Out of thirty monkeys (each immunized with a different Staphylococcal enterotoxoid) challenged intravenously with untreated Staphylococcal enterotoxin at 10 %kg., there was an illness response in only two monkeys. There were no deaths.

Out of twenty nine monkeys challenged intravenously with untreated Stephylococcal enterotoxin at 1000 r/kg., there were two deaths and fifteen illness responses. Twelve monkeys had no reactions.

The correlation between antibody titers and protection against intravenous challenge in these range finding studies was suggestive but not clear cut.

#### Extended Evaluation Studies

The following ten preparations were selected from the range finding studies for more extended comparisons of toxicities, antibody response, and degree and duration of immunity to intravenous challenge with untreated Staphylococcal enterotoxin:

Sample No.	Treatment
2	0.2% formalin at 37°C. for 2 weeks
10	0.2% formalin + 0.15% glycine at 25°C. for 2 weeks
12	0.3% hexamethylenetetramine at 37°C. for 3 weeks
14	0.2% beta-propiolactone at 37°C. for 2 hours
16	0.2% diethyl pyrocarbonate at 5°C. for 1 week
18	0.2% ethylene oxide at 5°C. for 2 hours
22	Exposure to methyl bromide vapor for 24 hours
25	1.0% sodium gluconate at 37°C. for 4 weeks
26	Exposure to ultraviolet light for 1 hour
28	Photodynamic action of methylene blue for 1 hour

Sets of twelve monkeys were immunized with each of the ten toxoid samples. The following table shows the toxicity responses to the first subcutaneous immunization injection of 0.10 mg. of toxoid with adjuvant:

Sample No.	Illness	Death	No Reaction
2	6	. 0	6
10	0	0	. 12
12	· 8	0	74
14	10	1	1
16	8	2	2
18	9	1	2
22	6 .	0	6
25	11	0	1
26	5	0	7
28	9	1	2

Sample #10 is the most impressive preparation to date. In a separate experiment, three monkeys were injected subcutaneously with 0.25 mg. of toxoid. There were no reactions. In addition to being the least toxic of the preparations tested, Sample #10 produced the highest antibody titers as measured by hemagglutination and gel-diffusion.

#### SUMMARY AND CONCLUSIONS

The range finding studies concerned with the preliminary evaluation of thirty Staphylococcal enterotoxoid samples have been completed.

Thirty pairs of rhesus monkeys, previously immunized with thirty different toxoid preparations, were challenged intravenously with untreated Staphylococcal enterotoxin three weeks after the third and final subcutaneous immunization injection.

Out of thirty monkeys (each immunized with a different Staphylococcal enterotoxoid) challenged intravenously with untreated enterotoxin at 10 v/mg., there was an illness response in only two monkeys. There were no deaths.

Out of twenty nine monkeys challenged intravenously with enterotoxin at 1000 r/kg., there were two deaths and fifteen illness responses. There were no reactions in twelve monkeys.

On the basis of toxicity, antibody titers, and intravenous protection results, ten Staphylococcal toxoid preparations were selected for more extended evaluation.

Sample #10 (0.2% formalin +0.15% glycine at 25°C. for 2 weeks) is the most impressive of the toxoid preparations on the basis of toxicity and antigenic response. The final evaluation, scheduled for May 30, 1965, will depend on the degree and duration of immunity to intravenous challenge with untreated Staphylococcal enterotoxin.

The attached summary sheets contain all the tabulated data available to date. Additional entries will be made as the experimental studies are extended.

#### DISTRIBUTION

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Semple: Control

Untreated Staphylococcal Enterotoxin, Lot No. 14-31

# 2

	89/6/6	9/9/63 9/12/63	9/23/63	10/7/63	9/23/63 10/7/63 10/14/63 11/8/63 11/18/63 12/2/63 12/10/63	11/8/63	11/18/63	12/2/63	12/10/63		
Monkey A						-					
Immunisation Dose, mg. (8q)	0.10				0.10		0.10				
Antibody Titer Hemagalutination	1			000		. 6	X	w w			
Gel-Diffusion Intravenous Challenge				No Zone		,		No Zone			
Dose, (mcg/kg.) Reaction									10.0 #.R.	-	
Monkey #2											
Immunization Dose, mg. (Sq) Reaction	0.25 X										
Monkey #40											
Immunitation Dose, mg. (SQ) Reaction		0.10	×								,
		$\left] \right]$								<b></b>	į

Illness (emesis, diarrhea) Death No Reaction

Sample #1

0.2% formalin at 37°C. for 1 week

	-	1		and the same and the same and the		·
	12/23/63			10.0 N.R.		1000 M.R.
	12 17:53 [12/23/63]		80,000 No Zone		9	No Zone
Date	12/9/63		0.25 N.R.		0.25 M.R.	
	10/23/63 11/25/63 12/9/63		10,000 No Zone			You Some
	10/23/63		0.25 N.R.		0.25 N.R.	
	9/18/63 10/15/63		< 10,000 No Zone		610 om	No Zone
	9/18/63		0.10 I		0.25 I	
		Monkey #47	Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge	Reaction Monkey #48	Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination	Gel-Diffusion Intravenous Challenge Dose, (mcg./kg.) Reaction

Sample 16

0.2% formalin at 37°C. for 2 weeks

12/23/63	10.0 M.R.	1000 I
12/11/63	1,280,000	1,280,000
१९/१/टा	0.25 M.R.	0.25 m.r.
10/28/63 11/25/63	320,000 100	320,000 240
10/28/63	0.25 M.R.	0.25 H.R.
9/24/63 10/21/63	160,000 204	320,000 190
8/54/63	0.10 7.8.	0.25 M.R.
Nonkey #49	Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey #50  Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

Sample #3

0.2% formalin at 37°C. for 3 weeks

12/31/63	10.0 M.R.	1000 I
12/23/63 12/31/63	2,560,000	<10,000 No Zone
12/9/63	0.25 M.R.	0.25 M.R.
12/3/63	2, <b>56</b> 0,000 159	20,000 No Zone
17/4/63	0.25 M.R.	0.25 N.R.
10/28/63	160,000 Positive	k 10,000 No Zone
10/1/63 10/28/63	0.10 N.R.	0.25 I
Honkey #55	<pre>Lumunization</pre>	Monkey #56  Irmunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

Sample 🌴

Sample #

0.25 formalin at 5°C. for 5 weeks

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1/24/64	8× 8×
10/21/63 11/18/63 11/2/63 11/25/63 12/23/63 12/30/63 1/10/54 1/24/64	347
22/30/6	о. 25 ж. ж.
12/23/63	320,000
11/25/63	0.25 N.R.
11/21/63	×
11/18/63	160,000 Positive
10/21/63	0.10 I
	Immunization Dose, mg. (S.,) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction Monkey #61 Immunization Dose, mg. (S.,) Reaction Antibody Titer Hemagglutination Gel-Diffusion

Sample #5

0.8% formalin at 37°C. for 1 week

# Pate

12/23/63	10.0 м.в.	1000 H.R.
१९/४८/टा	1,280,000	320,000 160
१९/६/उर	0.25 N.R.	0.25 m.m.
11/25/63 12/1/63 12/23/63	40,000 Positive	20,000 Positive
10/23/63	0.25 M.R.	0.25 M.R.
9/18/63 10/15/63	<10,000 Positive	<10,000 No Zone
9/18/63	0.10 I	0.25 M.R.
	Immunization Dose, mg. (Sq) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey Futh  Immunization Dose, mg. (Sq) Resction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

Sample #6

0.8% formalin at 37°C. for 2 weeks

	12/23/63	10.0 M.R.	1000 M.R.
	१९/१८७   १५/८७/हर	००० ००७ ००७	640,000 160
Date	१३/३/हर	0.25 M.R.	0.25 n.r.
	11/25/63	80,000 129	40,000 Positive
	10/28/63	0.25 M.R.	0.25 M.R.
	10/21/63	10,000 No Zone	< 10,000 No Zone
	8/sq/63	0.10 M.R.	0.25 N.R.
		Monkey #51  Infinitation Dose, mg. (9q) Reaction Autibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey #52  Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

Semple #7

0.8% formelin at 37°C. for 3 weeks

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12/31/63	10.0 M.R.	1000 I
12/23/63	20,000 No Zone	80,000 No Zone
12/9/63	0.25 M.R.	0.25 N.R.
12/3/63	<10,000 No Zone	80,000 Positive
11/4/53	0.25 N.R.	0.25 M.R.
10/1/63 10/28/63	< 10,000 No Zone	< 10,000 No Zone
10/1/63	0.10 M.B.	0.25 I
A C.	Monkey 57  Imaumization Dose, mg. (Sq) Reaction Antibody fiter Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Immunication Dose, mg. (SQ) Reaction Antibody Titer Heragglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

0.8% formalin at 5°C. for 6 weeks

Dute

1/24/54	1000 1	10.0 N.R.
1/10/24	2,550,000 Ito Zone	30,000 110 Zone
12/30/53	0.25 n.r.	0.25 II.B.
12/23/63	20,030 lio Zone	<10,000 Fo Zone
11/25/63	0.25 N.R.	0.25 N.R.
11/18/53	<b>410,000</b> No Zone	<10.000 Positive
10/21/53	0.10 I	0.2) I
C71	Irnunization Dose, mg. (S.) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (acg./kg.) Reaction	Monkey '53  Immunitation Dose, ag. (S.) Reaction Antibody Titer Hemagglutination jel-Diffusion Intravence Challenge Dose (ac./kg.) Reaction

Sample #9

0.2% formalin + 0.15% glycine at 25°C. for 1 week

12/23/63	10.0 M.R.	1000 I
12/9/63 12/11/63 12/23/63	1,256,000 380	160,000
	0.25 N.R.	0.25 <b>x. x</b> .
11/25/63	320,000 Positive	150,000 Positive
10/23/63	0.25 M.R.	0.25 N.R.
10/15/63	160,000 No <b>Zone</b>	<10,00€ No Zone
9/18/63	0.10 M.B.	0.25 I
10.00	Immunization Dose, mg. (SQ) Reaction Antibooy Titer Hemag'lutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey #42  Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

Sample #10

0.2% formalin + 0.15% glycine at 25°C. for 2 weeks

12/23/63	10.0	1000 H
12/17/63	640,000	320,000 50
12/3/63	0.25 N.R.	0.25 N.R.
11/25/63 12/3/63	160,000	40,000 Positive
10/28/63	0.25 N.R.	0.25 N.R.
10/21/63	10,000 No Zone	10,000 No Zone
9/24/63	0.10 I	0.25
Nonkey #53	Immunization Dose, mg. (S.) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey #54  Immunization Dose, mg. (S^) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

Sample #11

0.2% formalin + 0.15% glycine at 25°C. for 3 weeks

	1	
:	***************************************	
-		
[rz/31/63]	10.0 M.R.	1,000 I
12/23/63	320,000 No ¿one	80,000 No Zone
12/9/63	0.25 M.R.	0.25 M.R.
10/28/63 11/4/63 12/3/63 12/9/63 12/23/63 12/31/63	80,000 Positive	20,000 No Zone
11/4/63	0.25 N.R.	0.25 M.R.
10/28/63	< 10,000 No Zone	<10,000 No Zone
10/1/63	0.10 I	0.25 I
	Monkey #59  Immunitation Dose, rg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey #60  Immunization Dose, mg. (S:) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

Sample #12

0.3% hexamethylenetetramine at  $37^{\circ}$ C. for 3 weeks

12/10/63	10.0 N.R.	1000 M.R.
12/2/63	160,000	320,000
11/19/63	0.25 N.R.	0.25 %
11/8/63	160,000 Positive	320,000 100
10/16/63	0.25 N.R.	0.25 N.R.
10/7/63	160,000 No Zone	80,000 No Zone
6/11/63	0.10 N.R.	0.25 I
	Monkey #16  Immunization Dose, mg. (S.) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey #17  Immunization Dose, mg.(SQ) Reaction Antibody liter Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

1.0% hexamethylenetetramine at 37°C. for 3 weeks

		annesses an annesses
12/10/63	10.0 N.R.	1000 N.R.
12/2/63	80,000 Positive	80,000 Positive
11/19/63	0.25 N.R.	0.25 n.r.
9/11/63 10/7/63 10/16/63 11/8/63 11/19/63 12/2/63 12/10/63	320,000 305	160,000 Positive
10/16/63	0.25 n.r.	0.25 N.R.
10/7/63	80,000 No Zone	320,000 No Zone
89/11/63	0.10 N.R.	0.25 I
	Monkey #13  Immunization Dose, mg. (S2) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey #15  Immunitation Dose, mg. (S.) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

Sample #14

0.2% beta-propiolactone at 37°C. for ? hours

	·	
12/10/63	10.0 N.R.	1000 I
12/2/63 12/10/63	800,000 17 <sup>4</sup>	160,000 Positive
11/18/63	0.25 N.R.	0.25 N.R.
11/8/63	320,000 Positive	80,000 No Zone
10/14/63	0.25 N.R.	0.25 N.R.
10/7/63	20,000 No Zone	< 10,000 No Zone
9/10/63	0.10 I	0.25 I
	Monkey #3  Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey #4 Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

Sample /15

1.35 beta-propiolactone at  $37^{\circ}$ C. for 2 hours

	<u></u>	
12/10/63	10.0 N.R.	1000 M.R.
	80,000 Positive	80,000 Pesitive
11/18/63	0.25 N.R.	0.25 N.R.
11/8/63 11/18/63 12/2/53	80,000 Positive	160,000 Positive
10/14/63	0.25 N.R.	0.25 M.R.
£9/L/cī	< 10,000 No 20ne	<10,000 No Zone
9/10/63	0.10 I	0.25 I
	Intunization Dose, mg. (Sr) Reaction Antibody Titer Wencylluthation Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Linumization Dose, mg. (Sr) Reaction Antibody Titer Hemegglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

0.2% diethyl pyrocarbonate at 5°C. for 1 weak

# Pate

		•
12/10/63	10.0 M.R.	1000 H.R.
12/2/63	20,000 Positive	160,000 Positive
E9/81/TT	0.25 M.R.	0.25 M.R.
11/8/63	80,000 Positive	80,000 No Zone
£9/¼T/oT	0.25 M.R.	0.25 M.R.
10/1/63	80,000 213	20,000 No Zone
6/10/63	0.10 I	0.25 I
Monkey #11	Immunitation Dose, mg. (Sq.) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.)	Monkey #12  Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

Sample #17

1.0% diethyl pyrocarbonate at 5°C. for 1 week

12/23/63	10.0°	100 I
12/11/63	10,000 No Zone	20,000 ·
६९/६/टा	0.25 M.R.	0.25 M.R.
11/25/63	10,000 No Zone	10,000 No Zone
10/23/63	0.25 N.R.	0.25 M.R.
10/12/63	10,000 No Zone	10,000 No Zone
9/18/63	0.10 B.R.	0.25 M.R.
Monter #15	Immunization Dose, mg. (Sq) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey #46  Immunization Dose, mg. (SQ) Reaction Antibody Titer Benagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

Sample #18

0.2% ethylene oxide at 5°C. for 2 hours

## Dete

		•
2/10/63	10.0 M.R.	1000 H.R.
12/2/63 12/10/63	320,000 345	80,000 Positive
11/18/63	0.25 <b>M.</b> R.	0.25 M.R.
11/8/63 11/18/63	160,000 205	80,000 Pogitive
10/14/63	0.25 N.R.	0.25 M.R.
10/1/63	20,000	10,000 No Zone
8/10/63	0.10 I	0.25 I
ST.	Monkey #2  Immunitation Dose, mg. (SQ) Reaction Antibody Tiver Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey #10  Immunization Dose. mg. (S4) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

Sample #19

1.0% ethylene oxide at 5°C. for 2 hours

12/10/63	10.0 M.R.	1000
12/2/63	160,000 175	20,000 No Zone
11/19/63	0.25 M.R.	0.25 M.R.
11/8/63	160,000 Positive	410,000 No Zone
10/16/63	0.25 M.R.	0.25 H.R.
10/8/63	< 10,000 No Zone	4 10,000 No Zone
69/11/6	0.10 I	0.25 M.R.
	Monkey #22  Immunization Dose, mg. (84) Reaction Antibody Titer Hemagglutination Cel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey #23  Immunization Dose, mg. (3q) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

Sample #20

Exposure to ethylene oxide vapor for 24 hours

12/10/63	10.0 N.R.	1000 I
12/2/63	20,000 No Zone	20,000 No Zone
11/19/63	0.50 N.R.	0.50 N.B.
11/8/63	10,000 No Zone	40,000 No Zone
10/16/63	0.50 I	0.50 N.R.
10/8/63	10,000 No Zone	20,000 No Zone
8/m/63	0.10 M.R.	0.25 N.R.
	Fonter #20  Irmunization Dose, mg. (Sq) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey #21  Immunization Dose, mg. (Sq) Reaction Antibody Titer Hemagglutination Cel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

Sample #21

Exposure to ethyleneimine vapor for  $2^{\mu}$  hours

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ب	ı
r	1
$\boldsymbol{\vdash}$	1

12/10/63	10.0 N.R.	7000 1000
12/2/63	20,000 No Zone	< 10,000 No Zone
11/20/63	0.25 м.в.	0.25 M.R.
11/n/e3	< 10,000 No Zone	< 10,000 No Zone
10/11/63	0.50 M.R.	0.50 M.R.
10/8/63	10,000 No Zone	<10,00℃ No Zone
9/12/63 10/8/63	0.10 M.R.	0.25 M.R.
	Monkey #34  Immunization Dose, mg. (SC) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey #35  Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

Sample #22

Exposure to methyl bromide vapor for 24 hours

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J	e

12/10/63	10.0 N.R.	1000 I
12/2/63	160,000	10,000 Positive
11/18/63	0.25 M.R.	0.25 M.R.
11/8/63	140,000 No 2one	20,000 Positive
10/14/63	0.25 M.R.	0.25 M.R.
9/10/63 10/1/63	<10,000 No Zone	20,000 No Zone
9/10/63	0.10 N.R.	0.25 M.R.
Nonkey #1	Immunitation  Dome, mg. (SQ)  Neaction Antibody Titer  Hemagglutination Gel-Diffusion Intravenous Challenge Dome (mcg./kg.) Reaction	Monkey #8  Immunization Dose, ( mg./kg.) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

Sample #23

Exposure to propylene oxide vapor for 24 hours

107	8/11/6	10/8/63	10/16/63	11/8/63	11/19/63	12/2/63	12/10/63	The second secon
Monkey #24								
Immunization Dose, mg. (S2)	0.10		0.25		0.25			
Reaction	<b>-</b>		. X. X		. K.			
Antibody Titer Hemagglutination		10,000		10,000		20,000		
Gel-Diffusion		No Zone		No Zone		No Zone		
Intravenous Challenge							0.01	
Reaction							H.R.	
2								
Monkey #25							**************************************	
Immunization							<del></del>	
Dose, #g. (SQ)	0.25		0.25		0.25			
Reaction	н		M.W.		N.N.			
Antibody Titer				1				
Hemagglutination		8 8 8 9		86,88	-	320,000	-	
Gel-Diffusion		No Zone		Positive		Positive		
Intravenous Challenge								
Dose (mcg./kg.)							1000	
Reaction							<b></b>	
								,
	11							

# Methanol esterification

# Pats

12/10/63	10.0 I	1.0 M.R.
12/2/63	<10,000 No Zone	<10,000 No Zone
11/19/63	0.50 M.R.	0.50 ж.ж.
11/8/63	< 10,000 No Zone	<10,000 No Zone
10/16/83	0.50 M.R.	0.50 M.R.
10/8/63	< 10,000 No Zone	< 10,000 No Zone
69/11/6	0.10 M.R.	0.25 M.R.
	Monkey flb  Immunization Dose, mg. (Sq) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey #19  Immunization Dose, mg. (Sq) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

Exposure to ultraviolet light for 1 hour

2

12/10/63	10.0 E.R.	1000 I
12/3/63	320,000 Positive	640,000 No Zone
11/20/63	0.25 B.R.	0.25 M.R.
11/11/63	80,000 Positive	10,000 Positive
10/11/63	0.25 M.R.	0.25 H.B.
10/8/63	20,000 No Zone	1,0,000 160 Zome
8/21/6	0.10 I	0.25 M.R.
Menters 436	Dese, mg. (8q) Reaction Autibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey #31  Dese, mg. (Sq)  Reaction Antibody Titer  Hemagglutination Oel-Diffusion Intravenous Challenge Dose (mcg./kg.)  Reaction

Sample #26

Exposure to ultraviolet light for 1 hour

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Monkey #36	£9/2T/6	10/8/63	10/11/63	13/त/त	11/20/63	12/3/63	12/10/63	***
Immunitation Dose, mg. (SQ) Reaction Antibody Titer Benagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	20,000 No Zone	0.25 M.R.	80,000 Positive	0.25 M.R.	320,000 Positive	10.0 #.R.	
Monkey #37  Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Osl-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.25 M.R.	40,000 No Zone	0.25 N.R.	10,000 Positive	0.25 M.R.	640,000 No Zone	1000 I	

Exposure to ultraviolet light for 5 hours

12/10/63	10.0 H.R.	1000 I
12/2/63	320,000 No Zone	320,000 360 Zone
11/63 11/20/63	0.35 <b>#.</b> R.	0.25 %. %.
17/प/ध	10,000 Xo Zone	40,000 Fo Zone
10/17/63	0.50 #. #.	0.50 €
10/8/63	<ul><li>210,000</li><li>10 Zone</li></ul>	80,000 Fores
8/21/6	0.10 M.B.	0.25 m m
	Monkey #28  Immunitation Dose, mg. (84) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey #29  Immunization Dose, mg. (BQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

Photodynamic action of methylene blue - 1 hour

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12/10/63	10.0 M.B.	1000 H.R.
12/2/63	320,000 Positive	160,000 No Zone
11/20/63	0.25 M.R.	0.25 M.R.
11/11/63	10,000 No Zone	20,000 No Zone
10/11/63	0.25 M.R.	0.25 M.R.
10/8/63	160,000 No Zone	10,000 No Zone
9/12/63	0.10 I	0.25 N.R.
	Dose, mg. (Sq.) Reaction Autibody Titer Benegglutination Cel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey #31  Immunization Dose, mg. (Sq) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

Sample #29

Photodynamic action of methylene blue - 8 hours

12/10/63	10.0 M.R.	10000 I
12/2/63	40,000 No Zone	160,000 Positive
11/20/63	0.25 N.R.	. 0 . 25 . 34
१९/११/ए	∠10,000 ño Zone	10,000 No Zone
10/71/63	0.25 M.R.	0.25 M.R.
10/8/63	<10,000 No Zone	410,000 No Zone
9/21/6	0.10 I	0.25 I
	Immunization Dose, mg. (S.) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey #27  Immunitation Dose, mg. (32) Reaction Antibody Titer Hemagglutination :21-Diffusion In ravenous Challenge Dose (mcg./kg.) Reaction

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Semple #30

1

Acetylation with acetic anhydride

# 

		·
12/10/63	10.0 #.R.	1000 F.R.
12/3/63 12/10/63	160,000 Positive	80,000 No Zone
11/20/63	0.25 M.R.	0.25 F. F.
69/11/11   63/21/01	10,000 No Zone	10,000 No Zone
£9/11/ot	0.25 II.B.	0.25 ■.B.
10/8/63	10,000 No Zope	10,000 Fo Zone
69/21/6	0.10 I	0.25 I
	Immunisation Dose, mg. (SQ) Reaction Antibody Titer Benegglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey #39  Immunisation Dose, mg. (8q) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

3.2% formalin at 37°C. for 2 weeks

Date

	12/12/63 1/9/64	1/9/64	1/16/64	2/10/64	2/19/64	3/4/64	5/20/64	
Immunization Dose, mg. (Sw) Reaction* Antibody Titer Here jutination Jel-Diffusion Intravenció Challenge Dose (mg., kg.) Reaction	0.10 N.R.	40,000 No Zone	0.25 N.P.	<sup>1</sup> 0,000 No Zone	0.25 M.R.	80,000 No Zone	20,000 No Zone	
Lanunization  Dose, rg. (Sq)  Reaction Antibody Titer Hengglutination Jel-Diffusion Intravenous Ghallenge Dose (mcg./kg.) Resition	0.10 I	< 10,000 No Zone	0.25 N.R.	20,000 Positive	0.25 N.R.	80,000 No Zone	4 10,000 № Zone	,
* I, Illness (emesis, diarrhea	diarrhea)							

Semple #2

0.2% formalin at 37 C. for 2 weeks

2

	2/61/61	13/0/1 63/61/61	0/2//	L_					
	50/27/27	5/6/7	1/10/04	2/10/64	2/19/64	3/-1/64	5/50/64		
Monkey # 69								1	
Immulization								A	
Dose, mg. (SQ)	0.10		0.25		0.25				
Antibody Titer	. H. H.		. K.		œ.				
Hemagglutination Gel-Diffireion		410,000		20,000		320,000	4 10.000		
Intravenous Challenge		No Zobe		No Zone		Positive	No Zone		
Dose (mcg./kg.)								<del></del>	
Neaction									
Monkey \$70									
Immunization									
Dose, ng. (SQ)	0.10		0.25		0.25				
Reaction	н		M.R.		X.8.				
Antibody Titer									
Hemagglutination		€ 10,000		<b>1</b> 000		200 007	1.0		
Gel-Diffusion		No Zone		4, 44, 44, 44, 44, 44, 44, 44, 44, 44,	المالية	30,000	80,0		
Intravenous Challenge		2		PATTIENT		Positive	No Zone		
Dose (mcg./kg.)					يحدد				•
Reaction									
				i					
* I, Illness (emeste diameter)	At a washer								

0.2% formalin at 37°C. for 2 weeks

			,		
	5/20/64	20/2-1/	< 10,000 No Zone	80,000 No Zone	
	3/ 4/64		80,000 Positive	320,000 Positive	
	2/19/64		0.25 E. 25	0.25 M.R.	
	2/10/54		< 10,000 No Zone	80,000 Positive	
	1/16/64 2/10/54		0.25 M.R.	0.25 M.R.	
	1/9/64	æp.	< 10,000 No Zone	< 10,000 No Zone	
10000	12/12/63		0.10 I		(arrhea)
		Monkey # 71	Immunization  Dose, mg. (SQ)  Reaction*  Antibody Titer  Hemagglutination  Gel-Diffusion  Intravenous Challenge  Dose (mcg./kg.)  Reaction	Monkey # 72  Immunization Dose, mg. (Sq) Reaction Antibody Fiter Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	* I, Iliness (emesis, diarrhes)

Ti, Diness (emesis, diarrhea)
X, Death
N.R. No Reaction

0.2% formalin at 37°C. for 2 weeks

Date

0.10 N.R. A.10,000 No Zone No Zone No 2010  0.10  1  4.10,000 No Zone		12/12/63	12/12/63 1/9/64	1/16/64	2/10/64	2/19/61	2/13/64 3/ ·4/64	5/20/64	
0.10 I N.R. N.R. N.R. N.R. No. Zone No. Zone No. Zone	Immunization Drse, mg. (Sq) Reaction * Antibody Titer Hemacglutination Gel-Diffusior Intravencus Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	< 10,000 No Zone	0.25 74.75	80,000 Positive	0.25 N.R.	320,000 Positive	20,000 No Zone	
	Sonkey flu  Immunization Dose, mg. (Sq) Reaction Antibody Titer Henacclutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	< 10,000 No Zone	0.25 M.R.	4,10,000 No Zone	M M M	20,000 Nc Zone	< 10,000 No Zone	

Death No Reaction × × ×

0.2% formalin at 37°C. for 2 weeks

Pate

	19/6/1 89/21/21	1/9/6#	1/16/64	2/10/64	9/10/61	27.1.70		-	i
%onkey #75				10/0-1	7/17/04 3/-1/OF	3/-4/64	2/20/64		- 1
Immunization Dose, mg. (Sq) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (ncg./kg.) Reaction	0.10 I	4 10,000 No Zone	0.25 ##. #.	20,000 No Zone	0.25 M.R.	80,000 No Zone	< 10,000 No Zone		
Monkey 476  Immunization	0.10 M.R.	. 10,000 No Zone	0.25 <b>≭</b> .R.	10,000 No Zone	0.25 #.8.	20,000 No Zome	< 10,000 No Zone		•
* I, Illness (emesis, diarrhea	diarrhea)								

Death No Reaction

0.2% formalin at 37°C. for 2 weeks

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5/20/64	160,000 No Zone	640,000 No Zone
3/.4/64	320,000 Positive	2,560,000 2 <sup>1</sup> 40
2/19/64	0.25 N.R.	0.25 N.R.
2/10/64	80,000 Positive	1,280,000
1/16/64	0.25 N.R.	0.25 M.R.
1/6/1	10,000 No Zone	160,000 Positive
19/6/1 [59/21/21	0.10 I	0.10 N.R.
	Monkey #77  Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey #78  Immunization Dose, mg. (Sq) Reaction Antibody Titer Henagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

0.2% formalin + 0.15% glycine at 25°C. for 2 weeks

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			$\left  \cdot \right $
6/24/64	160,000 To Zone	1,280,000 Positive**	
1/3		المرامي منت مسينه أحبب من في بسيده الربي بيراني المراجب بياني	
4/1/6	640,000 Positive	1,280,000	
B/24/9 4/1/9	(γ) ei ο ≡		
2/11/B 3/11/B	1,280,000	640,000 235	
2/11/64	0.25 ≅.¤.	0.25 E.B.	
2/10/64	160,000 No Zone	40,000 Positive	
1/14/64 2/10/64	0.10 M.R.	0.10 F.R.	diampes)
	Monkey # 152  Immunization  Dose, mg. (Sq)  Reaction * Antibody Titer  Remagglutination  Gel-Diffusion  Intravenous Challenge  Dose (mcg./kg.)  Reaction	Monkey \$ 153  Immunization Dose, mg. (Sq) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challange Dose (mcg./kg.) Reaction	* I, Illness (caesis, diarribes

<sup>\*</sup> I, Illness (emesis, diarrhas) X, Death H.R. No Reaction

<sup>\*\*</sup> Quantitative titer to be determined

0.2% formalin #0.15% glycine at 25°C. for 2 weeks

Dete

	1/11/64	19/01/2	2/11/64	3/11/64	3/24/64	19/1/1	6/24/64		
Monkey # 154 Immunization Dose, mg. (Sq) Reaction * Antibody Titer Henagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 7.3.5	< 10,000 No Zone	0.25 N.R.	160,000 No Zone	0.25 N.R.	160,000 No Zone	160,000 No Zone	·	·
Monkey # 155  Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	< 10,00∪ No Zone	0.25 N.R.	320,000 Positive	0.25 N.R.	320,000 Positive	320,000 Positive		
Hiness (emests	di gambas h								

0.2% formalin ~ 0.15% glycine at 25°C. for 2 weeks

Bete

	1/14/64	2/10/64	2/11/64	2/11/64 3/11/64	3/54/64	19/1/1	49/47/9	-	
Monkey # 154									
Immunization  Dose, mg. (SQ)  Reaction *  Antibody Titer  Henagglutination  Gel-Diffusion  Intravenous Challenge  Dose (mcg./kg.)  Reaction	0.10 N.R.	< 10,000 No Zone	0.25 N.B.	160,000 No Zone	0.25 M.R.	160,000 No Zone	160,000 No Zone		
Monkey # 155  Immunization Dose, mg. (SQ) Reaction Antibody Titer Henagglutination Gel Diffusion Intravenous Chailenge Dose (mcg./kg.) Reaction	0.10 N.R.	< 10,0∪ No Zone	0.25 N.R.	320,000 Positive	0.25 N.R.	320,000 Positive	320,000 Positive		
* I. Hiness (emeste Atamber)									

1, Liness (emesis, diarrhes) X, Death W.R. No Reaction

0.2% formalin + 0.15% glycine at 25°C. for 2 weeks

Date

49/4Z/9	80,000 No Zone	10,000 No Zone	
η9/2/η	640,000 Positive	20,000 Positive	
3/24/64	0.25 N.R.	0.25 N.R.	
3/17/64	40,000 Positive	< 10,000 Positive	
49/21/2	0.25 M.B.	0.25 A.R.	
	10,000 No Zone	4 10,000 No Zone	
1/14/64 2/10/64	0.10 N.R.	0.1C N.R.	d extrace
	Monkey #156  Immunization Dose, mg. (Sq) Reaction* Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey # 157  Immunization Dose, mg. (Sq.) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	* I. Tinese (meste discribes

\* I, Illness (emesis, diarrhea) X, Death N.R. No Reaction

0.2% formalin + 0.15% glycine at 25°C. for 2 weeks

4/2/F/F		
19/4/1	80,000 No Zone	640,000 Positive
3/24/64 3/24/64	160,000 Positive	1,280,000
3/24/64	0.25 M.R.	0.25 N.R.
3/17/64	10,000 Positive	640,000
2/17/64	0.25 N.R.	0.25 N.R.
2/12/64	< 10,000 No Zone	160,000 No Zone
1/11/64	0.10 N.R.	0.10 N.R.
	Monkey # 158  Immunization Dose, mg. (Sq) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey # 159  Immunization Dose, mg. (Sq) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

<sup>\*</sup> I, Illness (emesis, diarrhea)
X, Death
N.R. No Reaction

<sup>\*\*</sup> Quantitative titer to be determined

0.2% formelin + 0.15% glycine at 25 C. for 2 weeks

Date

6/24/64	160,000 Positive	1,280,000 Positive**
η9/1/η	160,000	00°,048 084
3/54/64	0.25 M.R.	0.25 M.R.
2/11/64 3/11/64	80,000 240	160,000 Positive
49/LT/S	0.25 N.R.	0.25 M.R.
19/11/2 19/11/1	20,000 Positive	40,000 No Zone
19/11/1	0.10 A.R.	0.10 N.R.
	Monkey # 160  Immunization Dose, mg. (Sq) Reaction * Antibody Titer Remagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey # 161  Immunization Dose, mg. (Sq) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challege Dose (mcg./kg.) Reaction

\* I, Illness (casis, diarrhea) X, Death N.R. No Reaction

\*\* Quantitative titer to be determined

0.2% formalin + 0.15% glycine at 25°C. for 2 weeks

		·
19/172/9	2,560,000 Positive**	80,000 No Zone
19/1/1	1,280,000	80,000 380
3/24/64	0.25 N.R.	0.25 N.R.
3/11/64	5,120,000 1 <b>200</b>	80,000
2/11/64	0.25 N.R.	0.25 N.R.
1/14/64 2/14/64	2,560,000	10,000 No Zone
1/14/7	0.10 N.R.	0.10 N.R.
	Monkey # 162  Immunization Dose, mg. (Sq) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey # 163  Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

F. I. Illness (emesis, diarrhea)
X, Death
N.R. No Reaction

<sup>\*\*</sup> Quantitative titer to be determined

0.3% hexamethylenetetramine at 37°C. for 3 weeks

Date

	12/13/63	12/13/63 1/10/64	1/11/64	2/10/64	2/20/64	3/5/64	5/20/64	
<pre>!:onkey j 81  Immunization Dose, mg. (Sq) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction</pre>	0.10 I	< 10,000 No Zone	0.25 M.R.	40,000 Positive	0.25 M.R.	150,000 Positive	80,000 No Zone	
Monkey 482  Immunization Dose, ng. (Sq) Reaction Antibody Titer Hemacclutination Cel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	<10,000 No Zone	0.25 N.R.	160,000 190	0.25 n.r.	320,000	320,000 No 20ne	
* T TI TINGER (								 _

\* I, Illness (emesis, diarrhea)
X, Death
N.R. No Reaction

0.3% hexamethylenetetramine at 37°C. for 3 weeks

Date

5/20/64	80,000 No Zone	20,000 No 7one
3/5/64	150,000 Positive	40,000 Positive
2/20/64	0.25 N.R.	0.25 N.R.
2/10/64	40,000 No Zone	40,000 Positive
19/21/1	0.25 N.R.	0.25 N.R.
1/10/64	4.10,000 No Zone	<10,000 No Zone
12/13/63 1/10/64	0.10 I	0.10 I
	Manufaction  Dose, ng. (Sq) Reaction* Antibody Titer Hema_glutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.)	Monkey #84  Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

0.3% hexamethylenetetramine at 37 C. for 3 weeks

Date

	12/13/63	12/13/63 1/10/64	1/11/64	2/10/54	2/20/64	3/5/64	5/20/64
Monkey # 85  Immunization Dose, mg. (Sq) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	01.0 I	20,000	0.25 n.r.	40,000 Positive	0.25 n.r.	80,000 Positive	160,000 Positive
Monkey 4 85  Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 % %	<10,∝ No Zone	0.25 N.R.	340,000 100	0.25 N.R.	5,120,000	1,280,000 Positive
4) Liness (coesis, diarrhes	diarrhea)						

FI, Illness (emesis, diarrhea X, Death N.R. No Reaction

**>** 

Sumple #12

3.3% hexamethylenetetramine at 37°C. for 3 weeks

Date

					٠.				·		
5/20/64			320,000 No 7one					40,000 No 7one	,		
3/5/84			<del>.5,</del> 120,000 330					80,000		·	
2/20/54		0.25 II.R.				0.25	N.R.	:	,		
5/10/64			150,000			,		40,000 No Zone			./
1/11/64		0.25 N.R.	ş			0.25	R.R.				
12/13/53 1/10/64			20,000 Positive					<b>4</b> 10,000			
12/13/53	1	0.10 I				01.0	<b>}</b>				
	florkey f 87	Dose, mg. (SQ) Reaction *	Menajglutination Gel-Diffusion Intravencus Chillenge	Dose (mc;./kg.) Reaction	Nonkey #58	Lumumization Dose, mg. (SQ)	Reaction Antibody Titer	Henagglutination Gel-Diffusion	Intravenous Challenge Dose (mcg./kg.) Reaction		

0.35 hexamethyle cretramine at 37°C. for 3 weeks

5/20/64	640,000 No Zone	320,000 No 7one
3/5/64	1,280,000	640,000 Pcs1t1.n
2/20/24	0.25 N.R.	0.25 N.R.
2/10/64	1,280,000	160,000 Positive
1/11/64	0.25 N.R.	0.25 N.R.
1/10/54	160,000	< 10,000 No Zone
12/13/53	0.10 N.R.	0.1 I
	Monkey & 89 Immunization Dose, mg. (Sq) Reaction * Antibody Titer Hema, glutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey # 91  Immunitation Dose, mg. (Sq.) Reaction Antibody Titer Henagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

\* I, Illness (emesis, diarrhea)
X, Peath
N.R. No Reaction

0.3% hexamethylunetetramine at 37°C. for 3 weeks

Jate

	5/20/64	160,000 No 70ne	40,000 No 70ne
	3/5/64	40,000 Positive	80,000 No Zone
	2/20/64	0.2% N.B.	0.2 N.R.
	2/10/64	40,000 Positive	
17/24/6	1/11/9#	0.25 N.R.	0.25 N.R.
	1/10/04	< 10,000 Pos.tive	< 10,000 No Zone
12/13/60	15/17/03	0.10 N.R.	0.10 I
		Manualisation  Dose, mg. (Sq.)  Reaction *  Antibody Titer  Hema_Clutination  Gel-Diffusion  Intravenous Chullenge  Dose (mcg./kg.)  Reaction	Monkey 493  Immunization Dose, mg. (Sq) Reaction Antibody Titer Hemacolutination Cel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

			`		
0/20/	6/25/64	80,000 No 70n <del>e</del>		160,000 No Zone	
1, 1976).	*C.70/±	40,000 Positive		80,000 Positive	
ग५/५८/६		0.25 N.R.		0.25 N.N.	
 19/31/6		< 10,000 No Zone		40,000 Positive	
2/18/54		0.25 3.3.3.		0.25 N.R.	
75/11/2		< 10,000 No Zone	< 1C,000		
1/15/54		0.10 I		0.10 I	diarrhen)
	"onkey / 155	Immunization  Dose, mg. (SQ)  Reaction *  Antibody Titer  Hemajjlutination  Gel-Diffusion  Intravenous Challenge  Dose (mcg./kg.)  Reaction	Nonkey # 157 Demunization	Lose, mg. (SQ) Reaction Antibody Titer Hemacclutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	* I, Illness (emesis, diarrhen

0.2% beta-propiolactone at 37°C. for 2 hourn

Date

	1/12/64	4//۳۱/۶	9/18/61	0/01/6	10,100,0			
	10/2-1-		=/ TO/ O#	3, 10/64	3/25/64 4/8/64	4/8/64	6/25/64	
Monkey 168  Inmunitation  Dose, mg. (SQ)  Reaction *  Antibody Titer  Hema_Glutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.)  Reaction	0.10 I		0.25 N.B.	40,000 Positive	0.25 N.R.	80,000 Positive	80,000 No Zone	
Monkey \$169  Immunization Dose, mg. (Sq.) Reaction Antibody Titer Hemagglutination Cel.Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	B°, OCO No Zone	0.25 M.R.	320,000 No Zone	0.25 M.R.	320,000 Positive	1,280,000 No Zone	
* I. Illness (emests diarrhen	diarrhen)							

		1							
	1/12/64	2/14/64	2/18/64	3/18/64	3/52/64	3/25/64 4/8/64	49/46/9		
Nonkey 110		40-10 <b>1</b> 11-1-1-1					20/1-10		
Immunization  Dose, mg. (SQ)  Reaction *  Antibody Titer  Hema, glutination  Gel-Diffusion  Intravencus Challenge  Dose (mcg./kg.)  Reaction	0.10 I	10,000 No Zone	0.25 N.R.	10,000 No Zone	0.25 M.R.	10,000 No Zone	20,000 No Zone	,	
Lumunization Dose, mg. (Sq) Reaction Antib.ly Titer Hengglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	2,560,000	0.25 M.R.	1,280,000	0.25 N.R.	1,280,000	2,560,000 Positive**		
, Illness (emesis, diarrhem)	diarrhea)					7			

1, illness (emesis, diarrhea X, Death N.R. No Reaction \*\* quantitative titer to be determined

Sample #14

0.2% beta-propiolactone at 37°C. for 2 hours

6/25/64	160,000 No Zone	320,000 No Zone
19/8/th	160,000 Positive	320,000 Fositive
3/22/64	0.25 M.R.	0.25 N.R.
3/18/64	40,000 No Zone	μο,οπο Positive
2/18/64	0.25 N.R.	0.25 N.R.
2/14/64	160,000 No Zone	< 10,000 No Zone
1/12/64	0.10 I	0.10 I
	Monkey #172  Immunization Dose, mg. (Sq) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Chailenge Dose (mcg./kg.) Reaction	Monkey # 173  Immunization Dese, mg. (SQ) Reaction Antibody Titer Henagglutination Cel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

Illness (emesis, diarrhen) Death No Reaction

0.2% beta-propiolactone at 37°C. for 2 hours

6/25/64		10,000 No 2ορε	40,000 No Zone			
т9/8/п		10,000 No Zone	160,000 No Zone			
3/25/64		0.25 M.R.	0.25 N.R.			
3/18/64		40,000 No Zone	80,000 No Zone			
2/18/64		0.25 N.R.	0.25 M.R.			
2/14/64		< 10,000 No Zone	40,000 No Zone			
1/15/64		0.10 I	0.10 I			
	Conkey of 174	Immunization Dose, mg. (Sq) Reaction * Antibo.y Titer Remacglutination Gel-Diffusion Intravencus Challenge Dose (mcg./kg.) Reaction	Monkey \$175  Irrunization Dose, ng. (Sq) Reaction Antibody Titer Henacclutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction			

0.2% beta-propiolactone at 37°C. for 2 hours

Date

			•					
6/25/64			< 10,000 No Zone			320,000 No Zone		
19/8/1			< 10,000 No Zone	i	,	320,000 Positive		
3/52/64		0.25 8			0.25 N.R.			
3/18/64			< 10,000 No Zone			320,000 No Zone		•
2/18/54		0.25 N.R.			0.25 N.R.			
 5/14/64			< 10,000 No Zone			< 10,000 No Zone		
1/12/64		0.10 I			0.10 I			
	Nonkey 1.175	Immunization Dose, mg. (SQ) Reaction *	Hema_Glutination Gel-Diffusion Intravenous Chullenge Dose (mcj./kg.) Reaction	Norkey # 177	Lununization Dose, ng. (SQ) Reaction Antibody Titer	Hemacclutination Gel-Diffusion Intravenous Challenge	Dose (mcg./kg.) Reaction	

_		
49/91/9	20,030 No Zone	40,000 No Zone
3/30/64	160,000 No Zone	160,000 No Zone
3/16/64	0.25 N.R.	0.25 N.R.
3/10/64	< 10,000 No Zone	< 10,000 No Zone
2/10/64	0.25 M.R.	0.25 n.R.
1/31/64	2 10,000 No Zone	< 10,000 No Zone
1/6/64	0.10 I	0.10 I
	Monkey 196  Immunization  Dose, mg. (SQ)  Reaction *  Antibody Titer  Hema_Elutination  Gel-Diffusion  Intravenous Challenge  Dose (mcg./kg.)  Reaction	Monkey 437  Emmunization Dose, mg. (Sq) Reaction Antibody Titer Henacclutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

Sample #16

0.2% diethylpyrocarbonate at 5 °C. for 1 week

Illness (emesis, diarrhea) Death No Reaction

0.2% diethylpyrocarbonate at 5°C. for 1 week

				•
47/7//7	0/07/0	< 10,000 No Zone	20,000 No Zone	
3/20/64		40,000 No Zone	40,000 Fo Zone	
3/16/64		0.25 N.R.	0.25 N.R.	
3/10/64		<10,000 No Zone	< 10,000 No Zone	
2/10/64		0.25 N.R.	0.25 N.R.	
1/31/64		< 10,000 No Zone	4 10,000 No Zone	
1/6/64		0.10 I	0.10 I	
	"onkey / 100	<pre>Imnunization     Dose, mg. (Sq)     Reaction * Antibody Titer     Hemagglutination     Gel-Diffusion     Intravencus Challenge     Dose (mcg./kg.)     Reaction</pre>	Monkey #101  Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemacclutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	* 7 * 111

0.2% diethylnyrocarbonate at 5°C. for 1 week

Date

19/91/9	80,000 No Zone	40,000 No Zone
3/32/64	2,560,000 245	320,000 Positive
3/16/64	0.25 N.S.	0.25 N.R.
3/10/64	320,000 Positive	< 10,000 No Zone
2/10/64	0.25 M.R.	0.25 N.R.
1/31/64	80,000 Positive	4 10,000 No Zone
1/6/64	0.10 M.R.	0.10 I
	Monkey # 102  Immunization Dose, mg. (Sq) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey # 103  Immunization Dose, mg. (Sq) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

Illness (emesis, diarrhea) Death No Reaction . т, к, к.в.

0.2% diethylpyrocarbonate at 5°C. for 1 week

(0/0/10)		
3/30/64		
3/16/64	0.25 N.R.	
3/10/64	640,000 Positive	
79/01/2	0.25 N.R.	0.25 *
1/31/64	320,000	10,000 No Zone
1/6/64	0.10 I	0.10 N.R.
	Monkey #104  Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravencus Chillenge Ibse (mcg./kg.) Fraction	Monkey and Dose of (SQ)  The action  Antibody Tite:  Hemanication  Gel-Diffusion  Gel-Diffusion  Heraylutination  Gel-Diffusion  Heraylutination  Heraylutination  Heraylutination  Heraylutination  Heraylutination  Heraylutination  Heraylutination

X, Deatr. N.R. No.Resetton

0.2% diethylpyrocarbonate at 5°C. for 1 week

Dete

	Illness (emesis, diarrhen)
	is. diarrheal
	18. diarrhan

Death No Reaction

0.2% ethylene oxide at 5 C. for 2 hours

٠			
	m9/0E/9	20,000 No 7.0ne	80,000 No Zone
	4/13/64	μ0,000 Positive	80,000 Positive
	3/30/64	0.25 N.R.	0.25 N.R.
	3/19/61	20,000 No Zone	80,000 Positive
	5/24/64	0.25 n.r.	0.25 N.R.
	19/11/2	< 10,000 No Zone	< 10,000 No Zcne
	1/20/64	0.10 I	00 I
		Monkey # 180  Immunization Dose, mg. (SQ) Reaction * Antibody Ti.er Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey # 181  Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

\* I, Illness (emesis, diarrhea)
X, Death
N.R. No Reaction

0.2% ethylene oxide at 5°C. for 2 hours

Date

	1/06/1		0/01/0					
	1/20/03/7	7/27/04	2/24/64	2/24/64 3/19/64	3/30/64	4/13/64	#9/0E/9	
Monkey # 182	· ·	-	سيد دخد اد					
Immunization  Dose, and. (*E1)  Readtion*  Antibody After  Henagglutimation  Gel-Diffination  Intraveruns Challenge  Dose (mcg./kg.)  Remation	0.10 I	< 10,000 No Zone	0.25 N.R.	10,000 No Zone	0.25 N.R.	20,000 Positive	80,000 Positive	
Monkey #183  Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	10,000 Positive	0.25 N.R.	40,000 Positive	0.25 N.R.	40,000 Positive	160,000 Positive	•
Illness (emesis, diarrhea	iamrheal				1			

Death
No Reaction

	1/20/64	2/14/61	2/01.7/01	17. 17.				
1	10/02/2		5/54/64	3/19/64	3/30/64	4/13/64	t9/0E/9	
	0.10 I	< 10,000 No Zone	0.25 n.r.	μ0,000 Pcsitive	0.25 N.R.	40,000 Positive	80,000 No 7one	
	0.10 I	4 10,000 No Zone	×					
Ť	Liness (chests, diam'hes)					T		

'I, Illness (emesis, diarrhea) X, Death N.R. No Reaction

0.2% ethylene oxide at 5 C. for 2 hours

Date

1	1	1
19/02/9	640.000 Pogitive	20,000 No Zone
4/13/64	20,000 Positive	40,000 Positive
3/30/64	0.25 N.R.	0.25 N.R.
3/19/64	40,000 No Zone	40,000 No Zone
79/47/2	0.25 N.R.	0.25 M.R.
79/11/2	< 10,000 Positive	✓ 10,000 No Zone
1/20/64	0.10 N.R.	0.10 I
	Monkey # 186  Immunization Dose, mg. (Sq.) Reaction * Antibody Titer Hema_Glutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey # 187  Immunization Dose, mg. (Sq.) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

0.2% ethylene oxide at 5°C. for 2 hours

				- <del> </del>			
19/08/9	20 105 10	•	320,000 No Zone			20,000 No 7one	
19/81/1	0 15-1		160,000 80			80,000 Positive	
3/30/64		0.25 M.R.			0.25 N.R.		•
3/19/64			160,000 Positive			46,000 No Zone	
2/24/64		0.25 N.R.			0.25 N.R.		
2/14/64			80,000 Positive			40,000 Positive	
1/50/64		0.10 I			0.10 I		
	Monkey / 188	<pre>Lumunization</pre>	Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monke: / 189	Immunization Dose, ng. (SQ) Reaction Antibody Titer	Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	111

0.2% ethylene oxide at 5°C. for 2 hours

Date

Exposure to methyl branide vapor for 24 hours

Date

			_
7/1/5	20,000 No 70ne	< 10,000 No 7one	
77177	40,000 Positive	< 10,000 % Zone	
3/31/64	0.25 и.я.	0.25 7.8.	
3/20/.27	20,000 No Zone	20,000 No 20ne	
2/25/54	0.25 	0.25 M.R.	
79/71/2	< 10,000 Fo Zone	< 10,000 No Zone	
1,21,6.	0.10 I	0.10 I	Marrhea)
	Honoration Dose, mg. (Sq.) Reaction * Antibody Titer Hemauglutination Cel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey # 195  Irmunization Dose, mg. (Sq) Reaction Antibody Titer Hemacolutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	+ J. Illness (emesis, diarrhea

Death No Reaction ×× ×

Exposure to methyl bromide vapor for 24 hours

Date

1/1/64	40,000 No 7one	
4/14/54	160,000 Positive	320,000 Positive
3/31/64	0.25 м.в.	0.25 N.R.
3/20/54	160,000 Positive	160,000 Positive
2/25/64	0.25 N.R.	0.25 N.R.
5/14/54	320,000 Positive	10,000 No Zone
1/21/64	0.10 N.R.	0.10 N.R.
	Monkey # 196  Immunization Dose, mg. (SQ) Reaction* Antibody Titer Hemacglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey # 197  Immunization Dose, mg. (SQ) Reaction Antibody Titer Heragglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

1/1/64		80,000 No 7.one	
4/10/64	×	160,000 Positive	
3/31/64	0.25 N.R.	0.25 N.R.	
3/50/64	< 10,000 No Zone	160,000 No Zone	
2/25/64	0.25 N.R.	0.25 N.R.	
79/11/2	< 10,000 No Zone	160,000 No Zone	
1/21/64	0.10 I	0.10 M.R.	4 2 4
	Monkey 1 200  Immunization Dose, mg. (SQ) Reaction * Antibody Titer HemacGlutination Gel-Diffusion Intravenous Challenge	Dose (m.g./kg.) Reaction  Monkey J 201  Immunization Dose, mg. (SQ) Reaction Antibody Titer Henagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	* [. T] ness (emeste Atente

Exposure to methyl bromide vapor for 24 hours

Pate

19/1/2	80,000 No 7one	< 15,000 No 7one
η9/ητ/η	160,000 70	20,000 No 20ne
3/31/64	0.25 N.R.	0.25 N.R.
3/20/64	20,000 No 200e	10,000 No Zone
2/25/64	0.25 N.R.	0.25 M.R.
49/41/2	10,000 No Zone	< 10,000 N° Zone
1/21/64	0.10 I	0.10 N.R.
	Manunization  Dose, mg. (SQ) Reaction * Antibody Titer Hemacglutination Gel-Diffusion Intravencus Challenge Dose (mcg./kg.) Reaction	Monkey # 203  Immunization Dose, mg. (Sq) Reaction Antibody Titer Henagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

Illness (emesis, diarrhea) Death No Reaction

Exposure to methyl bromide vapor for 24 hours

Date

	1,21.5-	-9/1-1/2	77 52/3	-) 38.8	<b>ग</b> ९/ रहे. इ	4/14/54	49/1/2	1
<pre>!fonkey if 204 Immunization Dose, mc. (Sq) Reaction * Antibody Titer Hemacglutination Gel-Diffusion Intravencus Challenge Dose (mcg./kg.) Reaction</pre>	0.10 N.R.	80,000 No Zone	0.8 8.85 8.85	20,000 No Zone	0.25 M.R.	20,000 No Zone	10,000 No 7one	
Monkey # 205 Immunization Dose, mg. (Sq) Reaction Antibody Titer Benacclutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 1	4 10.000 %c Zone	0.25 N.R.	20,000 No Zone	0.25 N.B.	20,000 No 20ne	300 C C C C	
* I, Libes (enesis distriber	di empleo)							

Illiness (emesis, diarrhea)
 X, Death
 No Reaction

1.0% sodium gluconate at 37°C. for 4 weeks

Date

101 101	6/23/64	• .	2,560,000 Positive**		160,000 No ?one	
11/5/611	10/6/1	:	1,280,000		640,000 125	
3/53/64 4/6/61		0.25			0.25 N.R.	
3/16/64			40,000 Positive		20,000 Positive	,
2/11/64		0.25*		i	N.B.	
1/13/54 2/10/64			<pre>&lt; 10,000 No Zone</pre>		10,000 No Zone	
1/13/54		0.10 I		(	OI:D	
	Sonkey / 138	Immunization Dose, mg. (SQ) Reaction *	Antibody Titer Hema_Glutination Gel-Diffusion Intravencus Challenge Dose (mcg./kg.) Reaction	Monkey #139 Immunization Dose, mg. (So)	Reaction Antibody Titer Henagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.)	Neact10B

<sup>\*</sup> I, Illness (emesis, diarrhea)
X, Death
N.R. No Reaction

<sup>\*\*</sup> quantitative titer to be determined

1.0% sodium gluconate at  $37^{\circ}$ C. for 4 weeks

Pete

-			
	6/23/64	000 (Ort	80,000 80,000
1.10.101	#0/o/#	640,000 No Zone	80,000 No Zone
3/03/61.	5/65/04	0.25 M.R.	0.25 # .8.
3/16/61	- C (CT (C	80,000 To Zone	20,000 ¥0 2.000
2/17/64	2/1=/-	0.25 M.R.	0.25 B.R.
2/10/64	1	< 10,000 No Zone	< 10,000 No Zone
1/13/64		0.10 I	0.10 I
	Sonkey # 140	<pre>Immunization     Dose, mg. (Sq)     Reaction * Antibody Titer     Hemagglutination     Gel-Diffusion     Intravenous Challenge     Dose (mcg./kg.)     Reaction</pre>	Monkey £141  Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

1.0% sodium gluconate at 37°C. for 4 weeks

Date

6/23/64	640,000 Positive	320,000 No 7one
49/9/4	640,000 190	640,000
3/23/64	0.25 N.R.	0.25 N.R.
3/36/64	160,000 Positive	1,280,000 Positive
19/11/2	0.25 M.R.	0.25 N.R.
19/01/z	<.10,000 No Zone	40,000 No Zone
1/13/64	0.10 I	0.10 M.R.
	Hamization  Dose, mg. (SQ)  Reaction *  Antibody Titer  Hema, glutination  Gel-Diffusion  Intravencus Challenge  Dose (mcg./kg.)  Reaction	Monkey # 143  Immunization Dose, mg. (52) Reaction Antibody Titer Hemacclutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

1.0% sodium gluconate at 37 C. for 4 weeks

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19/22/9	640,000 No 7one	<sup>1</sup> ,000 Mo 7,000
19/9/1	640,000 160	1,280,000 Positive
3/23,/64	0.25 N.R.	0.25 N.R.
3/16/64	80,000 No Zone	160,000 No Zone
2/11/64	0.25 N.R.	0.25 M.R.
2/10/64	2 10,000 No Zone	< 10,000 No Zone
1/13/64	0.10 I	0.10 I
	Image of 144.  Image, mg. (Sq.)  Reaction * Antibody Titer Hemagelutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey #145  Immunization Dose, mg. (SQ) Reaction Antibody Titer Henagglutination Cel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

1.0% sodium gluconate at 37°C. for 4 weeks

Date

6/23/64	640,000 Positive	160,000 No 7one
45/9/4	1,280,000	320,000 No Zone
3/23/64	0.25 n.R.	0.25 N.R.
3/16/64	80,000 Positive	40,000 No Zone
2/11/64	0.25 1.8.	0.25 N.R.
2/10/64	< 10,000 No Zone	< 10,000 No Zone
1/13/54	0.10 I	0.10 I
	Inmunization  Dose, mg. (SQ)  Reaction * Antibody Titer  Hemejglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.)  Reaction	Monkey # 147  Immunization Dose, mg. (SQ) Reaction Antibody Titer Henacclutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

Illness (emesis, diarrhen) Death No Reaction \* H,

3.18.54 4/1/54 6/18/64		0.25 n.R.	543,000 2,550,000 Positive Positive		0.25 X.R.	80,000 150,000 Fositive Positive	
6/18/6h	50 /OT /C		2,550,000 Positive				
			543,000 Positive			80,000 Fositive	
3.18.54				;	0.25 N.R.		
3/12/64			2,550,000 Positive			40,000 Positive	
2/21/5	•	0.23 7.8			0.25 M.R.		
1/31/64	ļ.,		320,000 Positive			<ul><li>✓ 10,000</li><li>No Zone</li></ul>	
275,52		0.10			0.10 M.R.		
	": onkey 7 124	Immunization Dose, mg. (Sq) Reaction * Antibody Titer Hena Flutination	Gel-Diffusion Intravencus Chillenge Dose (mcj./kg.) Reaction	12nkey # 125	<pre>Immunization</pre>	Hemacclutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	

Sample #26

Exposure to ultraviolet light for 1 hour

49/81/9	320,000 Positive	20,000 No Zone
49/1/4	80,000 Positive	80,000 Positive
3/18/64	0.25 M.R.	0.25 M.B.
3/12/64	160,000 Positive	320,000 Positive
2/21/5	0.25 N.R.	0.25 N.R.
1/31/64	< 10,000 No Zone	10,000 No Zone
1/8/64	0.10 N.R.	0.10 I
	Monkey / 126  Immunization  Dose, mg. (Sq)  Reaction *  Antibody Titer  Hemagglutination  Gel-Diffusion  Intravencus Challenge  Dose (mcg./kg.)  Reaction	Immunization Dose, mg. (SQ) Reaction Antibody Titer Henagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

Illness (emesis. diarrhea)
Death
No Reaction

	1/8/64	1/31/64	19/21/2	3/12/64	3/18/64	4/1/64	6/10/61		L
Honkey / 128						20/21	0/10/04		
Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.)	0.10 N.R.	20,000 Positive	0.25 N.R.	320,000 Positive	0.25 N.R.	320,000 No Zone	320,000 No Zone		
Reaction Monkey # 129									
Immunization Dose, mg. (SQ) Reaction Antibody Titer	0.10 I		0.25 M.R.		0.25 M.R.			·	
Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction		2 10,000 No Zone		160,000 Positive		80,000 Positive	40,000 No Zone		
* I, Illness (emesis, diarriben	dierrhen								

Death No Reaction

Exposure to ultraviolet light for 1 hour

Date

	1/8/64	יוא/ נצ/ נ	19/61/6	3/10/6),	3/38/61	1. 1. 1.61.	6/10/21	
		50/11	5/ 75/ 04	3/ 15/04	2/10/04	4/1/24	to/ot/o	- !
Honkey / 132								
Immunization Dose, ng. (SQ) Reaction *	0.10 N.R.		0.25		0.25			
Antibody Titer Hemajglutination Gel-Diffusion		< 10,000 No Zone		80,000 No Zone		80,000 Positive	< 10,000 No Zone	
Intravenous Challenge Dose (mcg./kg.) Reaction						:		
Monkey #133						2		
Lanunization								
Dose, mg. (SQ)	0.10		0.25		0.25			اللفية والمقات
Antibody Titer	X.		N.N.		N.R.	,		والمراجعين
Hemagglutination Gel-Diffusion		< 10,000 No 2006		320,000		320,000	320,000	
Intravenous Challenge		anor ou		POSTETAG		</td <td>Positive</td> <td></td>	Positive	
Dose (mcg./kg.)								
neaction								
								ر المناوطي
* [ T] hass (emests distributed	At ownhas							

19/81/9		160,000 Positive
49/1/4	40,000 Positive	160,000 Positive
3/18/64	0.25 N.R.	0.25 N.R.
3/12/64	80,000 Positive	40,000 Positive
2/12/54	0.25 N.R.	0.25 N.R.
1/11/64	20,000 No Zone	< 10,000 No Zone
1/8/64	0.10 I	0.10 N.R.
	Interpose of 134  Immunitation Dose, mg. (SQ) Reaction * Antibody Titer Hemajglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	Monkey \$135  Immunization Dose, mg. (Sq) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

I, Illness (emesis, diarrhea)
X, Death
N.R. No Reaction

Photodynamic action of methylene blue - 1 hour

Pete

## Pet

	*		
	6/17/64	80,000 Positive	320,000 Positive
	3/31/64	320,000 165	640,000 150
	3/11/64	0.25 N.R.	0.25 M.R.
	3/11/64	320,000 Positive	160,000 Positive
	15/11/2	0.25 N.R.	0.25 M.R.
	1/31/64	< 10,000 % Zone	80,000 Positive
10,7	1/1/27	0.10 I	0.10 I
		Manumization Dose, mg. (SQ) Reaction* Antibody Titer Hemagglutiration Gel-Diffusion Intravencus Challenge Dose (mcg./kg.) Reaction	Mose, mg. (Sq.)  Dose, mg. (Sq.)  Reaction  Antibody Titer  Hemagglutination  Gel-Diffusion Intravenous Challenge  Dose (mcg./kg.)  Reaction  * I. Illness (emenia dierrhea)

\* I, Illness (emesis, diarrhea)
X, Death
N.R. No Reaction

Photodynamic action of methylene blue - 1 hour

Date

49/11/9	160,000 Positive	320,000 Positive
3/31/64	1,280,000	640,000 115
3/17/54	0.25 n.r.	0.25 4.R.
3/11/54	1,280,000 Positive	83,300 Positive
45/11/2	0.25 	0.25 N.R.
1/31/54	80,000 Positive	10.000 No Zone
19/2/1	0.10 N.R.	0.10 I
	Manufaution Dose, mg. (Sq) Reaction * Antibody Titer Hemacglutination Cel-Diffusion Intravencus Challenge Dose (mcg./kg.) Reaction	Monkey 4 115  Irrumization Dose, mg. (SQ) Reaction Antibody Titer Heragglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

3/11/64 3/31/54	0.25 II.R. 1.280,000	0.25 N.R. 0 640,000 ive 140
2/11/54 3/11/54	0.25 N.R. 320,000 Positive	0.25 M.R. 540,000 Positive
4 1/31/54	10,000 Positive	80,000
1/1/2	Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemacglutination Gel-Diffusion Intravencus Challenge Dose (mcg./kg.) Reaction	Immunization Dose, mg. (Sq.) Reaction Antibody Titer Hemucclutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

Photodynamic action of methylene blue - 1 hour

Dete

	49/21/9		640,000 Positive	320,000 Positive	
	3/17/54 3/31/54		150,000	30,000 Positive	
	3/11/54		0.25 R.		
10/ 25/2	3/11/5₩		1,280,000 Positive	150,000 Positive	
12/44/6	±6/π/2		0. H R. S.	0.25 8. 8.	
1/31/2	50/50/1		10,300 Positive	60,000 No Zone	
1/4/2	7,		0.10 E.E.E.E.E.E.E.E.E.E.E.E.E.E.E.E.E.E.E.	о С С	Larrhea)
	-	Sonkey 1 115	Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hencgclutination Gel-Diffusion Intravencus Challenge Dose (mcg./kg.) Reaction	Emunization Dose, mg. (SQ) Reaction Antibody Titer Henacclutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	* I, Illness (enesis, diarrhea)

Death No Reaction

Photodynamic action of methylene blue - 1 hour

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Bet

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١	19/21/9		160,000 No Zone
	3/31/64		80,000 % Zone
	3/17/64		0.25 M.R.
	3/11/64		160,000 Positive
	19/11/8 19/11/8		0.25 M.R.
	1/31/64		1,0,000 No Zone
	19/2/1	0.10 x	0.10 I
		Immunization  Dosc, mg. (Sq)  Reaction * Antibody Titer  Hemagglutination Gel-Diffusion Intravencus Challenge Dose (mcg./kg.)  Reaction	Immunization Dose, mg. (Sq) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction

1, Libers (emesis, distribes)
X, Death
N.R. No Reaction